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Molecular Analysis of the *gat* Genes from *Escherichia coli* and of Their Roles in Galactitol Transport and Metabolism

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In enteric bacteria, the hexitol galactitol (Gat) (formerly dulcitol) is taken up through enzyme II (II^{Gat}) of the phosphoenolpyruvate-dependent phosphotransferase system (PTS), and accumulated as galactitol 1-phosphate (Gat1P). The *gat* genes involved in galactitol metabolism have been isolated from the wild-type isolate *Escherichia coli* EC3132 and cloned on a 7.8-kbp *Pst*I DNA fragment. They comprise six complete open reading frames and one truncated open reading frame in the order *gatYZABCDR′*. The genes *gatABC* code for the proteins GatA (150 residues) and GatB (94 residues), which correspond to the hydrophilic domains IIA^{Gat} and IIB^{Gat}, and GatC, which represents a membrane-bound transporter domain IIC^{Gat} (35 kDa, 427 residues). The three polypeptides together constitute a II^{Gat} of average size (671 residues). Gene *gatD* codes for a Gat1P-specific NAD-dependent dehydrogenase (38 kDa, 346 residues), *gatZ* codes for a protein (42 kDa, 378 residues) of unknown function, and *gatY* (31 kDa, 286 residues) codes for a D-tagatose-1,6-bisphosphate aldolase with similarity to other known ketose-bisphosphate aldolases. The truncated *gatR′* gene, whose product shows similarity to the glucitol repressor GutR, closely resembles a *gatR* gene fragment from *E. coli* K-12. The *gat* genes map in both organisms at similar positions, in *E. coli* K-12, where they are transcribed counterclockwise at precisely 46.7 min or 2,173 to 2,180 kbp. The genes are expressed constitutively in both strains, probably due to a mutation(s) in *gatR*. Transcription initiation sites for the *gatYp* and the *gatRp* promoters were determined by primer extension analysis.

In enteric bacteria, the polyhydric alcohol galactitol (Gat) (formerly dulcitol) is transported and phosphorylated through a phosphoenolpyruvate (PEP)-dependent galactitol:phosphotransferase system (PTS) (II^{Gat}) (9, 10). During uptake of a PTS carbohydrate, a phosphoryl group is transferred by the general PTS proteins enzyme I (EI) (gene *ptsI*) and histidine protein (HPr) (gene *ptsH*) from PEP to the substrate-specific enzymes II (EIIs) (for a review, see reference 13). The phosphoryl group is first accepted by the hydrophilic domain IIA, then by IIB. In a third step it is transferred to the substrate located in the membrane-bound transporter IIC, and the substrate phosphate is released into the cytoplasm. The three domains of an EII may be fused into a single protein, or they can be separated into two or three distinct proteins.

During galactitol uptake through II^{Gat}, galactitol 1-phosphate (Gat1P) is generated and converted by an NAD-dependent Gat1P-dehydrogenase (gene *gatD*) into D-tagatose 6-phosphate (Tag6P) (9, 10, 20). Further metabolism requires phosphofructokinase I (gene *pfkA*) to generate D-tagatose 1,6-bisphosphate. This kinase can be substituted for by phosphofructokinase II (gene *pfkB*) in strains overexpressing the enzyme, which is normally expressed at a low level in *Escherichia coli* K-12 (5). Finally, the bisphosphate is hydrolyzed by a ketose-bisphosphate aldolase into dihydroxyacetone phosphate and glyceraldehyde phosphate. The aldolase was claimed to be heat labile, conferring a Gat(Ts) phenotype to all Gat^+ strains including Lederberg's strain (10). Seven genes involved in galactitol metabolism have been cloned from the chromosome of a wild-type isolate (EC3132) of *E. coli* and found to cluster in a *gat* operon (*gatYZABCDR′*). Based on sequence similarities the following functions were assigned to these genes: *gatY*, tagatose-1,6-bisphosphate-aldolase; *gatABC*, subunits of a II^{Gat}; *gatD*, galactitol-1-phosphate-dehydrogenase; *gatR*, fragment of a repressor. A function could not be assigned to GatZ, nor was a gene with similarity to known hexose or phosphohexose kinases found (12). In this paper we present results which identify the gene products encoded in the *gat* operon and their function in galactitol metabolism, locate the transcription initiation site for *gatYp* and *gatRp*, and allow the precise mapping of the *gat* genes in *E. coli* K-12.

MATERIALS AND METHODS

Chemicals. [^{3}H]galactitol was obtained from ICN Biomedicals; L-[^{35}S]methionine and ^{35}SATP were from Hartmann Analytic, Braunschweig, Germany. All other chemicals were of commercial origin.

Bacterial strains and plasmids. The properties of the strains and plasmids used are given in Table 1. Derivatives of plasmid pBNL6 are shown in the corresponding figures together with the results.

Culture media and growth conditions. Lennox broth (LB), phosphate-buffered minimal medium (MM), and the MacConkey agar plates containing 1% (wt/vol) of the carbohydrate to be tested have been described before (8, 9). In minimal media, the following were added to the concentrations indicated: L-amino acids and nucleosides, 20 mg/liter, carbohydrates 10 mM, vitamins 5 mg/liter. Sterile filtered antibiotics were used at the following final concentrations (milligrams per liter): kanamycin and chloramphenicol, 25; tetracycline, 10; ampicillin, 100; rifampin, 400. Growth on media containing galactitol or D-glucitol was at 30°C.

Enzyme assays. Assays for galactitol uptake or EII^{Gat} phosphorylation activity tests using isolated membrane vesicles and membrane-free extracts have been described previously (9). Assays for aldolase activity were done in 50 mM KCl–50 mM Tris-HCl (pH 7.5) with 2.4 mM D-tagatose 1,6-bisphosphate as a substrate, 0.2 mM NADH, and 1 U of each triose phosphate isomerase and glycerin phosphate dehydrogenase. After equilibration of the assay mixture at the desired temperature the reaction was started by adding extract from exponentially grown cells disrupted by ultrasonic treatment. The decrease of NADH was measured photometrically and compared to a control without substrate. Protein concentrations were determined according to the method of Bradford (4). Activities are given in nanomoles per minute per milligram of protein.

Genetic and cloning techniques. Conjugations involving pULB113 and its derivatives were performed according to the method of van Gijsegem and Toussaint (18) using auxotrophy and antibiotic resistance markers to counterselect against the parent strains. All exconjugants were purified before further testing. Cloning and isolation of DNA was done by the standard methods described by Sambrook et al. (16) and Ausubel et al. (1). Restriction enzymes and other

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TABLE 1. Bacterial strains and plasmids used

Strain or plasmid	Relevant genotype[a] or phenotype and parent strain	Reference or source
Strains		
E. coli EC3132	Wild type, prototroph, *gat*$^+$ Δ(*atl-rtl*) Str^s	3
E. coli K-12		
CA8000	HfrH *gatR49* Gat$^-$Kba(Ts)	1
JM109	*gatR49* Gat$^-$ *recA1* Kba(Ts)	1
JWL153	*gut-52 gatR49* Str^r Kba(Tr)	9
JWL193	JWL153 *gatD51*	10
JWL194	JWL193 *gat-54*	10
JWL194BN	JWL194 *recA1 gut300*::Tn*10* Tc^r	This study
JWL146-1	*gut-50 gat-50 gatR49 rpsL104* Kba(Ts)	9
LLR101	*gat*$^+$ *gatR49* Δ(pts::Tn*5*) Kba(Ts)	R. Lux
DF1010	Δ(*rha-pfkA*)*200* Δ*pfkB201*	5
E. coli C	Wild type, prototroph, Δ*gat atl*$^+$ *rtl*$^+$	
K. pneumoniae KAY2026	Δ*gat dal*$^+$ *rbt*$^+$	19
Plasmids		
pULB113	Kn^r Ap^r Tc^r Tra$^+$	18
pSU18	Cm^r	1
pBluescript SK$^-$/KS$^-$	Ap^r	1

[a] Alleles *gat-50*, *gat-54*, *gut-50*, and *gut-52* inactivate galactitol or glucitol transport, respectively, while *gatR49* (formerly *gatC* [for control]) yields an inactive repressor. Genes for D-arabinitol and ribitol utilization are abbreviated *atl*/*rtl* for *E. coli* and *dal*/*rbt* for *K. pneumoniae.*

enzymes used in recombinant DNA experiments were used according to the specifications of the manufacturers.

Southern experiments and T7 overexpression of gene products. For Southern hybridization experiments, the DNA labeling and detection kit and the DIG luminescent detection kit from Boehringer Mannheim were used according to the protocols of the manufacturer. The T7-dependent overexpression of gene products described by Tabor and Richardson (17) was performed according to the following protocol. Cells of strain JM109 harboring the plasmid pGP1-2 and the cloned fragment in either pBluescript SK$^-$ or -KS$^-$ were grown overnight at 30°C in Lennox broth with kanamycin and ampicillin. The overnight cultures were diluted to 2×10^8 cells per ml and grown to 10^9 cells per ml. For each assay, 2 ml of cells was washed in minimal medium, resuspended in 4 ml of minimal medium with glucose (0.2%) containing Difco Met assay medium (0.2%), and incubated at 30°C for 60 min. The cells were shifted to 42°C for 20 min, rifampin was added, and the cells were incubated for an additional 20 min at 42°C. Half a milliliter of the cells was labelled with 1 μCi of L-[^{35}S]methionine at 30°C for 10 min. After centrifugation, the pellet was resuspended in 100 μl of cracking buffer (1). Aliquots of 20 μl were treated for 5 min in boiling water before they were separated by SDS-PAGE.

Primer extension. The described primer extension experiments were done according to the method of Ausubel et al. (1). The labeled oligonucleotides were purified with MicroSpin Sephacryl HR resins from Pharmacia.

RESULTS AND DISCUSSION

Cloning of the *gat* genes from *E. coli* EC3132. The *gat* genes were cloned from the wild-type isolate EC3132 by using plasmid pULB113, which carries mini-Mu phages (18). R′gat$^+$ derivatives were crossed with the Gat$^-$ strain JWL194BN (*gat-54 gatD51 recA1* Tc^r) to select for Gat$^+$ exconjugants. The DNA from one R′gat$^+$ plasmid was used for shotgun cloning experiments, and various fragments were cloned into pSU18 (Fig. 1). Strains JWL193 (*gatD51*) and JWL194BN were transformed and plated on MacConkey galactitol plates. Among several thousand white transformants, three Gat$^+$ colonies were found. JWL193/pBNL5 (3.3-kbp *Eco*RI fragment) showed weak fermentation, while JWL194BN/pBNL6 (7.8-kbp *Pst*I fragment) and JWL193/pBNL7 (11.45-kbp *Pst*I fragment) showed strong fermentation. Due to the inherent instability of these plasmids in all strains, enzyme assays and complementation experiments were done only with freshly transformed cells. To differentiate between chromosomal DNA of EC3132 and pULB113 DNA, five hybridization probes (A to E in Fig. 1) were used on EC3132 chromosomal DNA which had been treated with different restriction endonucleases (data not shown). The results of these hybridization and restriction analyses are summarized in Fig. 1.

Identification of the genes for galactitol transport and the Gat1P dehydrogenase. Strains like *E. coli C* and *Klebsiella pneumoniae* KAY2026 which lack all *gat* genes were complemented by pBNL6 to a fully Gat$^+$ phenotype, but not by pBNL5. This plasmid, which carries only genes *gatA′B*$^+$*C*$^+$*D*$^+$, could, however, complement mutants lacking dehydrogenase (JWL193), transport (JWL146-1), or both activities (JWL194) (Table 2 and data not shown). Transport mutants complemented by pBNL5 consistently showed lowered transport. This is due either to the lack of the genuine *gatYp* promoter or,

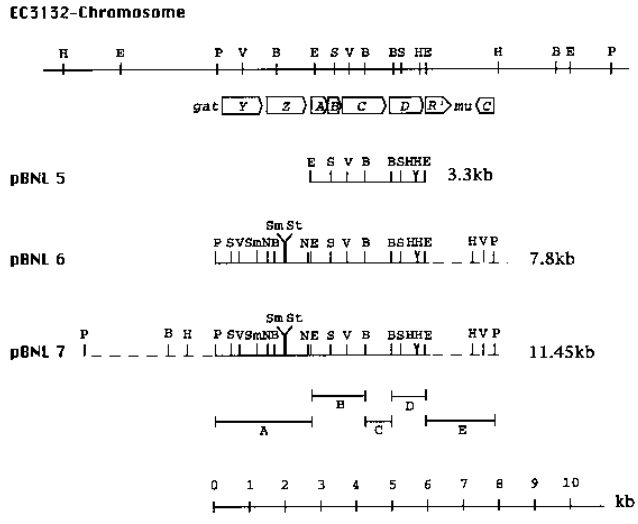

FIG. 1. Restriction map of the plasmids pBNL5 to -7. The dashed lines represent DNA derived from plasmid pULB113. The restriction map of the chromosome of *E. coli* EC3132 was deduced from hybridization experiments with probes A to E derived from pBNL6. The eight identified open reading frames are shown schematically. The *gatR′* fragment (68 residues) is not drawn to scale. Abbreviations: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; N, *Nru*I; P, *Pst*I; S, *Sph*I; Sm, *Sma*I; St, *Stu*I; V, *Eco*RV. Further information is given in the text.

TABLE 2. Galactitol transport and Tag-1,6-BP aldolase activities from various strains[a]

Strain	+	Plasmid	Galactitol transport		Tag-1,6-BP aldolase	
			−Gat	+Gat	−Gat	+Gat
JWL141	*gat*$^+$		12.0	10.3	≤1.0	≤1.0
JWL141	*gat*$^+$	+ pBNL6			34.0	
JWL153	*gat*$^+$ Kba(Tr)		15.4	13.5	28.0	49.0
JWL153	*gat*$^+$ Kba(Tr)	+ pBNL6			215.0	234.0
E. coli C	Δ*gat*		≤0.1	≤0.1	≤0.1	≤0.1
E. coli C	Δ*gat*	+ pBNL6			87.0	52.0
KAY2026	Δ*gat*		≤0.1	≤0.1	4.0	≤1.0
KAY2026	Δ*gat*	+ pBNL5	0.8	0.8	4.0	
KAY2026	Δ*gat*	+ pBNL6	9.5	6.5	390.0	133.0

[a] Tag-1,6-BP, D-tagatose 1,6-bisphosphate. Cells were grown exponentially in LB (no glucose) at 30°C with or without galactitol (0.2%) plus the appropriate antibiotic for at least 4 h after induction. [^{3}H]galactitol (25 μM) transport activity and Tag-1,6-BP aldolase activity tested at 2.4 mM Tag-1,6-BP are given in nanomoles per minute per milligram of protein.

more likely, to a partial deletion of *gatA* truncated for its 135 5′-terminal base pairs. Several deletions, in particular pBNL9Δ20B and pBNL9Δ12B (GatD$^+$) and pBNL9Δ22A (GatD$^-$), allowed a more precise mapping of *gatD* and of the transport genes *gatABC*, located immediately upstream (Fig. 2).

According to the DNA sequence of the pBNL6 insert (12), *gatD* codes for a protein (346 amino acids) with similarity to other polyalcohol dehydrogenases and a characteristic NAD-binding domain. To identify the various *gat* gene products, several subclones were tested by using the T7 polymerase/T7 promoter overexpression system (17). SDS-polyacrylamide gels from cells overexpressing the genes consistently showed three peptides related to *gat* and one peptide encoded downstream of the *gat* genes. This protein (25 kDa) represents the repressor MuC of bacteriophage Mu as shown by the sequencing data. Its structural gene, which is transcribed divergently from the *gat* genes (Fig. 3, lanes 1 to 3), has apparently been cloned from pULB113, as is often found with this system (18). The protein band corresponding to GatD showed an apparent molecular mass of approximately 38 kDa (Fig. 3, lanes 6 and 9 to 12), in good agreement with the calculated mass (M_r 37,422) deduced from the amino acid sequence.

Genes *gatABC*, located immediately upstream of *gatD*, are related to the transport and phosphorylation of galactitol. Gene *gatC* apparently codes for a very hydrophobic protein (427 amino acids) with similarity to the IIC^{Fru} transporter domain of the fructose PTS. It thus represents most likely the membrane-bound IIC^{Gat} transporter. GatC shows on gels an apparent molecular mass of 35 kDa (Fig. 3, lanes 9 to 12), which is significantly smaller than the calculated value (M_r 45,600). This behavior is typical for strongly hydrophobic proteins and is also seen for the truncated GatC of pBNL9Δ5A (Fig. 3, lane 8) (26 instead of 33 kDa) and of pBNL9ΔA (Fig.

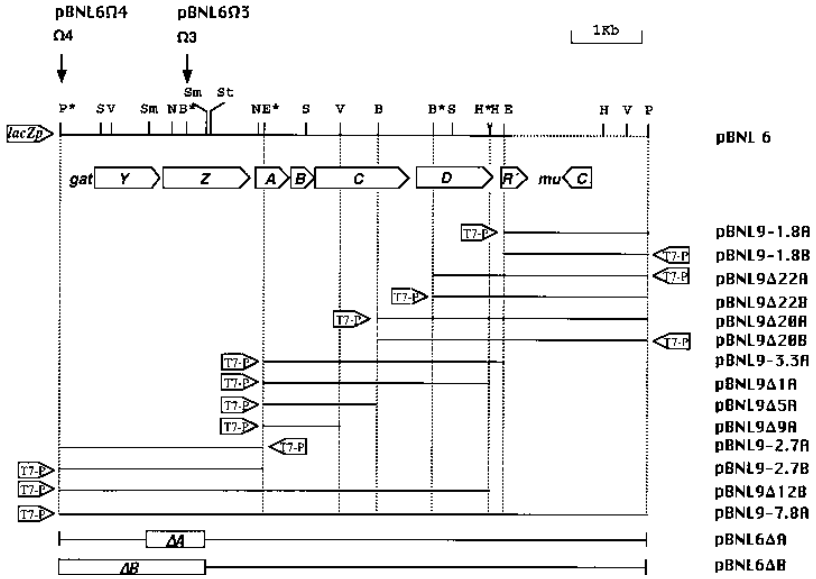

FIG. 2. Plasmids (series pBNL9) for the complementation and T7 overexpression experiments. The fragments derived from pBNL6 were cloned into pBluescript SK$^-$ (A) or KS$^-$ (B). The positions of the T7 promoter (T7-P) and the *lacZ* promoter (*lacZp*) of the vector are indicated. The boxes ΔA and ΔB represent the deleted DNA of pBNL6. The two insertion points of the omega element are indicated by arrows, and restriction sites conserved between *E. coli* EC3132 and K-12 are indicated by asterisks. For further explanation, see Fig. 1.

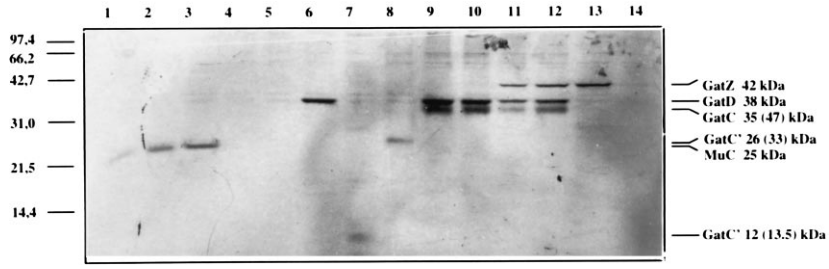

FIG. 3. Autoradiogram of radiolabeled overproduced gene products of the *gat* operon from *E. coli* EC3132. The protein standard is given on the left (in kilodaltons), and the apparent molecular masses of the identified gene products are indicated on the right (in case of a deviation from the molecular mass deduced from the amino acid sequence, this is additionally indicated in parentheses). Lane 1, pBNL9-1.8B; lane 2, pBNL9Δ22A; lane 3, pBNL9Δ20B; lane 4, pBNL9-1.8A; lane 5, pBNL9Δ22B; lane 6, pBNL9Δ20A; lane 7, pBNL9Δ9A; lane 8, pBNL9Δ5A; lane 9, pBNL9Δ1A; lane 10, pBNL9-3.3A; lane 11, pBNL9Δ12B; lane 12, pBNL9-7.8A; lane 13, pBNL9-2.7B; lane 14, pBNL9-2.7A.

3, lane 7) (12 instead of 13.5 kDa). The genes *gatAB* encode two small hydrophilic peptides (GatA [150 amino acids] and GatB [94 amino acids]) which do not show similarity to other IIA/IIB domains of the PTSs. For unknown reasons, the proteins could not be detected on gels, but their essential role in transport and phosphorylation was evident from the complementation tests mentioned before (Table 2). Cells which express II^{Gat} constitutively but lack the enzymes for the degradation of D-arabinitol (Atl) show an Atl^s phenotype, while cells which lack II^{Gut} for D-glucitol uptake and phosphorylation but are able to metabolize D-glucitol 6-phosphate become Gut^+ through II^{Gat} activity (9). Strains JWL146-1 and JWL194, which lack II^{Gat} and II^{Gut} activities and have a Gut^- Atl^r phenotype become Gut^+ Atl^s after transformation with pBNL5 and pBNL6, further indicating that II^{Gat} activity is encoded on both plasmids.

As expected for a galactitol PTS encoded in the *gat* operon, neither cell extracts from strain LLR101 [K-12 Δ(*pts-crr*) gat^+], which lacks EI and HPr but expresses the *gat* genes constitutively, nor extracts from the Δ*gat* strains showed any PEP-dependent galactitol phosphorylation. This contrasts with the activity in a Gat^+ strain, e.g., JWL153 (5.8 nmol of Gat1P formed per min per mg of protein). By using washed membrane and supernatant fractions from wild-type cells and from the various mutants, it could be shown that this PEP-dependent galactitol phosphorylation required not only EI, HPr, and membranes from gat^+ strains (supplying IIC^{Gat}) but also soluble components encoded in genes *gatA* and *gatB* (data not shown). These results support the conclusion that GatA and GatB correspond to the soluble domains IIA and IIB of a galactitol PTS (II^{Gat}), which together with GatC would comprise 671 amino acids. As yet, we have not been able to identify which of GatA and GatB corresponds to IIA^{Gat} and which corresponds to IIB^{Gat}.

Identification of further *gat* genes and gene products. Two further open reading frames (*gatY* and *gatZ*) are located upstream of the transport genes within the *gat* operon (Fig. 1). GatY resembles several ketose-bisphosphate aldolases, notably the D-fructose-bisphosphate aldolase (Fda) of *Bacillus subtilis* (38% identical residues). Similar to this aldolase, GatY is shorter than other aldolases (286 instead of around 370 residues), lacking three internal blocks of ca. 25 residues each (near residues 54, 88, and 252). GatZ (378 amino acids), however, does not show any similarity to other proteins (except for AgaZ, see below), in particular not to sugar or sugar-phosphate kinases.

In T7 overexpression experiments, a protein that corresponds to GatZ (42 kDa), but none that corresponds to GatY (calculated value, 31.5 kDa), could be seen (Fig. 3, lanes 11 to 13). This is surprising because a very high Tag-1,6-bisphosphate aldolase activity is expressed from pBNL6 (Table 2) and from truncated plasmids (e.g., pBNL9-2.7B) encoding only *gatY* and *-Z*. To test this activity, D-tagatose 1,6-bisphosphate and cell extracts from Δ*gat* strains which either have a low endogenous activity (KAY2026) or lack it completely (*E. coli* C) before transformation were used. Gat^+ strains of *E. coli* K-12 are unable to grow at temperatures above 30°C on galactitol as sole carbon source or on a mixture of glycerol and galactitol (10). Cells of *E. coli* C transformed with pBNL6 retain this phenotype. Cells of *E. coli* K-12 have been claimed to express a (heat-) labile aldolase which apparently corresponds to GatY (6, 9). Derivatives (e.g., JWL153 in Table 2) able to grow on galactitol or in the presence of galactitol at 42°C carried mutations in the gene locus *kba* (for ketose-bisphosphate aldolase), located near *argG* (71.4 min) (9). These express a high Tag-1,6-BP aldolase activity (Table 2) with an increased thermal stability (6) which can suppress the Gat(Ts) phenotype of normal strains and allow growth at high temperatures. By using a set of Tn*10* insertions and physical mapping, the Kba(Tr) mutation could be mapped to 70.7 min (of the new map) or 3,300 kbp (unpublished results). This corresponds to a newly described *aga* locus (for *N*-acetyl-galactosamine degradation) with genes *agaY* and *agaZ*, which encode two open reading frames with good similarity to GatY and GatZ (54 and 53% identical residues, respectively) (15). The nature of the suppression has yet to be determined.

By first inserting the omega DNA element (14) into the *Bam*HI site of *gatZ* and then removing it by *Hin*dIII treatment (pBNL6ΔΩ3 in Fig. 2), the reading frame of *gatZ* was destroyed without preventing expression of the genes located downstream. Mutants transformed with pBNL6ΔΩ3 became Gat^+ when they expressed GatZ in *trans* (e.g., JWL193 and JWL194), while the Δ*gat* strains vary in phenotype. KAY2026/pBNL6ΔΩ3 shows a fully Gat^+ phenotype; *E. coli* C/pBNL6ΔΩ3 shows a weakly positive phenotype. Two deletions (Fig. 2) which both remove the amino-terminal end of GatZ and either the carboxy-terminal part (pBNL6ΔA) or the entire *gatY* gene (the remaining *gat* genes expressed from *lacZp*) were also tested. Both deletions can again be complemented in JWL193 and JWL194, but not in the Δ*gat* strains. Obviously, the lack of GatZ causes a decrease in galactitol fermentation which can be suppressed by a *gatZ* copy expressed in *trans* and by an unidentified gene in KAY2026. The corresponding activity is perhaps related to the ability of KAY2026/pBNL6 to grow on galactitol at all temperatures, whereas *E. coli* C/pBNL6 shows a Gat(Ts) phenotype. No tagatose-6-phosphate kinase activity related to GatZ could be detected, and strain DF1010 (Δ*pfkA* Δ*pfkB*)/pBNL6 retains a Gat^- phenotype. This result and the lack of similarity

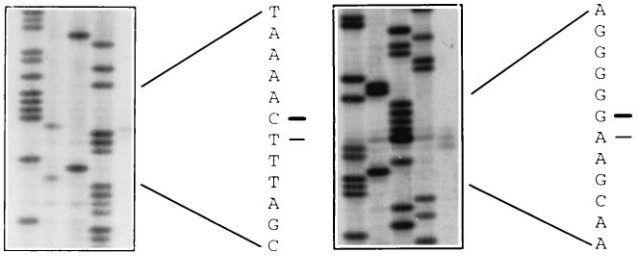

FIG. 4. Primer extension signals for *gatYp* and *gatRp*. The DNA sequence shown is the complementary strand of the mRNA. Parts of the *gatY* (left) and *gatR* (right) sequences are given together with the detected primer extension signals. The bars indicate the strong and weak primer extension signals.

of GatZ with other ketose-phosphate kinases argue against GatZ being a kinase as postulated (15). The similarity between GatZ and AgaZ, however, argues for an essential role in their respective metabolism.

Identification of regulatory elements involved in *gat* expression. Insertion of the omega DNA element of Prentki and Krisch (14) into the polylinker site of pBNL6 (pBNL6Ω4 in Fig. 2) did not affect the Gat^+ phenotype of transformants (data not shown). Clones, however, with the element inserted into the *Bam*HI site of *gatZ* (pBNL6Ω3) were unable to complement $GatD^-$ mutants with (JWL193) and without (JWL194) transport activity or the Δ*gat* strains, i.e., they were unable to express genes located downstream of the insertion. Because JWL193 and JWL194 express *gatY* and *gatZ* in *trans*, an essential promoter must be located between the two omega elements (Fig. 2). A primer extension experiment was performed with a 30-mer primer complementary to bp 31 to 61 behind the initiator ATG of GatY. A strong signal which begins 24 nucleotides (at G-376) and a weak signal 23 bp upstream of the putative translational start (residues 406 to 409 of the published sequence) could be detected (Fig. 4). A protein (31 kDa) has been isolated from *E. coli* K-12 whose amino-terminal sequence (MYVVTKQMLN) corresponds to the open reading frame of GatY beginning with nucleotides 406 to 409 (6a). This result corroborates the conclusion that we identified G-376 correctly as the transcriptional start site (+1). Consequently, a −35 consensus (TcGACA) separated by 17 bp from a −10 consensus (cATcAT) could be identified. This consensus is separated by 5 bp from the transcription initiation site (+1), by 24 bp from a ribosome binding site (AGGAt), and by 35 bp from the initiation codon.

Sequencing revealed downstream of *gatD* the 5′ end of the open reading frame *gatR* coding for 88 amino acids (12). Its first 68 residues include a putative helix-turn-helix DNA-binding motif (residues 24 to 43) and resemble (33% identical residues) the amino-terminal part of GutR, the repressor of the *gut* operon from *E. coli* K-12. The remaining 20 residues must have derived through an artificial fusion from pULB113 vector DNA. The GatR part is the truncated form of a highly similar (98% identical residues) open reading frame of 187 residues which maps in *E. coli* K-12 at 46.7 min (2,180 kbp) of the genome (15a). The corresponding sequence had been identified as the IS*3E* insertion site by Komoda et al. (7) (GenBank accession number X64217-8) and by Umeda and Ohtsubo (GenBank accession number D12598). Recently, we cloned a functional copy of *gatR* from a strain with an inducible *gat* operon, on a 1.2-kbp fragment. It complements Gat^+ strains of *E. coli* K-12 to an inducible phenotype. We sequenced the region immediately surrounding the putative (according to Umeda and Ohtsudo) IS*3E* insertion site (Thr-99). We found the same reading frame as for the K-12 gene and high identity (86% identical residues) up to residue 105, except for Thr-99 not being present in the new sequence. From position 106 to position 118 the reading frames deviate (unpublished results). We are currently repeating the cloning and sequencing of the IS*3E* insertion sequence from strains of *E. coli* K-12 with and without IS*3E* in the *gatR* locus, and we are cloning and sequencing the complete *gatR* genes from these strains and from *E. coli* EC3132 to find out whether these differences are due to chromosomal rearrangements or to sequencing errors.

Within the intergenic region between *gatD* and *gatR* (5 bp after the stop of *gatD*), a putative transcription termination structure is seen which constitutes a perfect 9-bp palindrome (CGGGCCAGCgtgatGCTGGCCCG). The AT-rich region which often follows termination loops could not be detected. Primer extension experiments with a 30-mer primer (complementary to bp 29 to 58 of *gatR*) revealed a signal indicating C-5836 (strong band) and perhaps T-5837 (weak band) as the transcription initiation site (+1) for *gatR*, 35 bp upstream of the initiation codon for GatR (Fig. 4). No clear promoter consensus but a putative ribosome binding site (TATGGG) is located in front of *gatR*.

All *gat* genes are present in *E. coli* K-12. Using probe B (Fig. 1) in hybridization experiments, the *Eco*RI fragment of pBNL5 (genes *gatA′B*$^+$*C*$^+$*D*$^+$ *gatR′*) could be detected in the chromosomes of EC3132, of the Gat^+ strain JWL153, and of the Gat^- strain CA8000 (HfrH), while in the Gat^- strain JM109 a shortened fragment (2.7 kbp) was found. No signal could be detected in the Δ*gat* strains *E. coli* C and *K. pneumoniae* KAY2026 in which the *atl-rtl* (*dal-rbt*) genes have replaced (genotypic exclusion) the *gat* genes (11, 21). With probe A (genes *gatYZA′*), identical signals could be detected in EC3132, JWL153, CA8000, and JM109, but no signals could be detected in *E. coli* C and KAY2026. Thus, the conservation of several restriction sites between the *gat* genes from EC3132 and *E. coli* K-12 (Fig. 2) together with the enzyme tests and the complementation experiments clearly indicate the presence of a complete and highly similar *gat* operon in *E. coli* K-12. It should be located at precisely 46.7 min (2,173 to 2,180 kbp) of the gene map and transcribed counterclockwise in this organism.

Similar to *E. coli* K-12, strain EC3132 expresses the *gat* genes constitutively as confirmed by enzyme (data not shown) and by Northern hybridization tests using probe C (Fig. 1). The levels of *gat* mRNA were identical for uninduced and induced cells of *E. coli* K-12 (e.g., JWL153) and of EC3132 (data not shown). This appears to be due to the presence of a mutation (*gatR49*) in the original K-12 strain (10). In some strains, e.g., W3110, MG1655, BHB2600, EJ1315, HB101, and an early *E. coli* K-12, an IS*3E* insertion copy is found, but not in others, e.g., EC3132 (2, 7) (unpublished results). The allele *gatR49*, as mentioned before, can be complemented by a $gatR^+$ allele to an inducible phenotype in strains of *E. coli* K-12 and in EC3132, thus indicating the presence of an inactive repressor in these strains. It remains to be shown how far this is due to the IS*3E* insertion or to other (e.g., frameshift) mutations within *gatR*.

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